



## Effect of charcoal rot on selected putative drought tolerant soybean genotypes and yield

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### ABSTRACT

Charcoal rot (CR), caused by the fungus *Macrophomina phaseolina* (Tassi) Goid. is a pervasive disease of economic significance on soybeans (*Glycine max* (L.) Merr.). Similarly, drought is the leading cause of yield loss in soybean worldwide. In this study, CR severity and seed yield were determined in irrigated and non-irrigated environments in 2011, 2012, and 2013 for thirteen soybean genotypes, nine of which were previously determined to be drought tolerant (DT). The objectives were to determine the severity of CR in the putative DT genotypes and to determine the over-all effect of CR on yield in irrigated and non-irrigated environments. Colony Forming Units (CFUs) at reproductive stage R7 were used to assess disease severity and classify each genotype's response to CR. A stress tolerance index (STI) was used to determine the relative impact of soil moisture stress (i.e. no irrigation) on the 13 genotypes. Over all three years in both irrigated and non-irrigated environments, five genotypes were consistently rated as moderately resistant to CR (MRCR) and three genotypes were consistently rated as susceptible to CR (SCR), whereas the responses of the remaining five genotypes varied between MRCR and SCR. Averaged over the three years, there was a wide range (0.36–1.09) of STI values among genotypes. Even though there was a consistent trend, there was a very weak relationship between STI and CFU's at the R7 growth stage. Regression analysis indicated that as CFUs at R7 increased, seed yield decreased, although the relationship was not significant in every year and irrigation environment. Nonetheless, across all years and irrigation environments, a pooled (global) slope indicated a yield loss of 11.5 kg ha<sup>-1</sup> for every 1000 CFUs at R7. These data indicated that as CFUs at R7 increased, seed yield decreased in both irrigated and non-irrigated environments. However, as the relationship between CR disease severity and DT was minimal, it may be necessary to select for resistance to both traits using environments where both soil moisture stress and CR are high.

### 1. Introduction

Soybean [*Glycine max* (L.) Merr.] is a major oilseed crop produced and consumed worldwide. Charcoal rot (CR), caused by the fungus *Macrophomina phaseolina* (Tassi) Goid., is a pervasive disease of economic significance on soybeans that generally occurs when plants are under heat and drought stress (Wrather and Koenning, 2006; Mengistu et al., 2015). Charcoal rot, a biotic stress (Mengistu et al., 2011), is therefore impacted by drought, an abiotic stress (Sinclair et al., 2007). Soybeans are constantly exposed to a variety of biotic (i.e., infection by pathogens) and abiotic (i.e., high or low temperature, too much or too little water, salinity, and mineral deficiency/toxicity) stresses that adversely affect their growth, metabolism, and yield (Sinclair et al.,

2010). Charcoal rot's impact and prevalence in the US and worldwide have already been reported (Mengistu et al., 2015).

In the context of this study, 'soil moisture stress' (SMS) is interchangeably used to mean the conditions under non-irrigation (rain-fed). Soil moisture stress may occur often or occasionally during the season, depending on the frequency and the amount of precipitation. According to the definition of the National Drought Mitigation Center, drought is a protracted period of deficient precipitation resulting in major and significant yield loss (<http://drought.unl.edu/>). Drought conditions may happen rarely in comparison to SMS under rain-fed environments. Since the severity of CR increases with increased SMS (Mengistu et al., 2015), experimental measurement ensures that the desired environmental moisture conditions are established for accurate disease measurement

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and yield. Soil moisture stress is measured using water tension, which is the suction that roots exert to extract water from the soil and is sometimes referred to as suction or matric potential. Thus, soil water tension is a good indicator of water stress and is measured in pressure units such as kilopascals (kPa) or centibars (cbars). Generally, for sandy loam soils when the soil water tension in the entire root-zone is between 50 and 80 kPa, irrigation needs to be initiated. Under such water tension, leaves start to show symptoms of water stress (Smiley, 1999).

Even though disease incidence of charcoal rot has been reported to have increased over the last 30 years throughout the north central regions of the United States (Birrenkott et al., 1984; Bradley and Rio, 2003; ElAraby et al., 2003; Yang and Navi, 2005), the impact of CR on suppressing soybean yields is more pronounced in Arkansas, Kansas, Kentucky, Missouri, Mississippi, and Tennessee than in the northern states, with an estimated yield loss averaging  $2.7 \times 10^7$  tons per year from 1996 to 2007 (Wrather and Koenning, 2009), making charcoal rot the most damaging soybean disease in the southern United States.

Although the contribution of charcoal rot towards yield loss in soybean has been estimated as above, it has not been well defined. Yield loss estimates have been complicated due to the confounding effect of SMS and charcoal rot, which has made it difficult to make recommendations to growers for managing charcoal rot. When varying levels of SMS occur from year-to-year, its overall impact on yield will also vary (Bowen and Schapaugh, 1989; Gray et al., 1991; Manici et al., 1995; Mengistu et al., 2011). Such variation further compounds the problem of objectively separating and determining the contributions of both SMS and disease severity towards yield loss.

In a previous report by Mengistu et al. (2011) the extent of yield reduction from charcoal rot was estimated by removing the effect of SMS using irrigation and methyl bromide (soil fumigant). In that study (Mengistu et al., 2011), the infection rate of *Macrophomina phaseolina* in the soil was significantly reduced and yield was increased by 6–30% due to irrigation and fumigation treatments alone. Application of irrigation is generally recommended as one of the management tools to reduce yield losses associated with CR disease (Grau et al., 2004). However, recent research indicates that disease development was favored under irrigation in infested fields more than previously thought (Mengistu et al., 2011). In contrast, in the same study Mengistu et al. (2011) also indicated that yield loss under non-irrigated and fumigated plots was difficult to measure because of the confounding effects of SMS and CR severity.

Soybean genotypes with putative DT have been released as public and commercial varieties (Chen et al., 2007a, 2007b; Devi et al., 2014; Shannon, unpublished data). In the southern United States for example, soybean breeding programs conducted by the University of Arkansas, Fayetteville, and the USDA-ARS in Raleigh, NC, have been developing drought-tolerant genotypes for more than 20 years (Tommy Carter, personal communication) under non-irrigated environments. These efforts have involved the traditional approach of mating parents with drought-tolerant characteristics and then evaluating and identifying progeny under field conditions.

A drought index, used in the selection process, was calculated by dividing yield under drought by yield under irrigation and then multiplying by 100 (Pathan et al., 2014). The genotypes with the least amount of wilting and the highest drought index were selected based on the techniques published by Pathan et al. (2014) and by selecting the drought tolerance associated with slow wilting and  $N_2$  fixation (Devi et al., 2014). Even though some moderately resistant germplasms were released, no breeding effort has yet been expended on combining drought tolerance with charcoal rot resistance.

The resistance of soybean genotypes to *M. phaseolina* is often measured by determining the colony forming units (CFU) of the fungus in sub-surface roots and lower stem tissue (Mengistu et al., 2007, 2013; Paris et al., 2006) and then calculating the area under the disease progress curve (AUDPC) based on the CFU. This method has also been used to determine CR resistance among common bean genotypes

(Pastor-Corrales and Abawi, 1988). The availability of disease-resistant genotypes has been utilized in many crops, including soybean (Danielson et al., 2004; Mengistu and Grau, 1987; Mengistu et al., 2013; Paris et al., 2006; Rabedaux et al., 2005; Smith et al., 2010), to assess the impact of disease on yield relative to susceptible genotypes under various sets of environments. Similarly, simultaneously measuring the relative CR resistance and yield of drought tolerant genotypes (Chen et al., 2001, 2007a, 2007b; Sloane et al., 1990), compared to genotypes with moderate resistance to CR (MRCR) and those susceptible to CR (SCR), could provide understanding for how these two traits vary together under irrigated and non-irrigated situations. Knowing the yield potential of DT genotypes, relative to that of genotypes with moderate resistance or susceptibility to CR in irrigated and non-irrigated treatments would be critical information for growers. If growers knew that they were losing yield to CR when using DT cultivars, they would be better equipped to weigh the benefits and risks of planting DT genotypes in CR infested soils. The objectives of this study were to determine the CR severity and yield of putative DT genotypes and their overall effects under irrigated and non-irrigated environments.

## 2. Materials and methods

### 2.1. Field plot design and treatments

A field study was conducted from 2011 through 2013 at the West Tennessee Research and Education Center at Jackson Tennessee (35.65N latitude 89.51W longitude). The field in which the study was conducted has a known history of high CR disease pressure. The experimental design was a randomized complete block design with a split-split plot with four replications. Maturity groups (MG) were main plots, soybean genotypes within maturity group were the sub-plots, and irrigation was the sub-sub-plot treatment.

The experimental field had been under no-till management with continuous soybean cropping for over 10 years prior to the initiation of the experiment. A week prior to planting each year the field trial site (with a dimension of 97.54 m wide x 42.67 m long) was divided into six grids, and 10 soil cores were collected per grid in a zigzag pattern to determine the colony forming units per gram of soil (CFU  $g^{-1}$ ). The CFU  $g^{-1}$  of soil of *M. phaseolina* ranged from 300 to 1000 CFU  $g^{-1}$  of soil. Inoculum distribution of *M. phaseolina* remained within the same range throughout the field in all three years. The planting dates were 9 May in 2011 and 2012, and 14 May in 2013.

The resistance to charcoal rot of nine putative DT genotypes with unknown CR resistance was assessed along with two moderately resistant (MRCR) and two susceptible (SCR) genotypes with unknown drought tolerance (Table 1). The thirteen genotypes were from two maturity groups, six MG IV and seven MG V. There were two groups of putative DT genotypes. The first group of DT genotypes (cvs. Dyna-Gro 36C44, Progeny 4408, Trisoy 4788, USG 75Z38 and USG Allen) was from Dr. Grover Shannon (University of Missouri, unpublished data). These genotypes were selected from 40 commercial varieties that were tested for drought tolerance during 2008 and 2009 at the University of Missouri-Delta Research Center, Clarkton, MO. The genotypes with the least amount of wilting and the highest drought index (genotypes were scored for leaf wilting during the stress period, and yields were assessed at the end of the growing season and used to calculate a drought index) were selected based on the techniques published by Pathan et al. (2014). The second group of DT genotypes was 'Osage', (Chen et al., 2007a), R01-581F (Chen et al., 2007b), R02-1325 (Devi et al., 2014), and R07-7232 (derived from the cross of R97-1634 x PI 471938). The drought tolerance associated with slow wilting and  $N_2$  fixation was derived from Jackson, PI 227557, R07-7232 and PI 471938 (Devi and Sinclair, 2013; Devi et al., 2014; Sinclair et al., 2007). Two genotypes with unknown DT but with known resistance to CR namely: DT97-4290 (MG IV, Paris et al., 2006) and DS-880 (MG V, Smith et al., 2010) were

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